American Journal of Hematology 58:145–147 (1998)

Cloning and Sequencing of an Alternative Splicing-Derived cDNA Variant of the GM-CSF Receptor Alpha Subunit, Which Encodes a Truncated Protein

Xiaotang Hu[1] and Kenneth S. Zuckerman[2]*

[1,2]Department of Internal Medicine, Division of Medical Oncology and Hematology, University of South Florida, and H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

[2]Department of Biochemistry and Molecular Biology, Division of Medical Oncology and Hematology, University of South Florida, and H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

GM-CSF interacts with the low affinity GM-CSF receptor α-subunit, which leads to high affinity association with the α-subunit/common β-subunit complex and transduction of intracellular signals leading to proliferation, differentiation, and/or activation of hemopoietic cells, predominantly in the neutrophil and monocyte/macrophage lineages. Several alternative splicing-derived variants of the GM-CSF receptor α-subunit have been described previously by this and other laboratories. A newly discovered alternative-splicing derived variant was isolated from the peripheral blood mononuclear cells of a patient with juvenile myelomonocytic leukemia. This variant lacks 397 base pairs corresponding to exons 8–11 of the wild type GM-CSF receptor α-subunit cDNA and potentially encodes a 233 amino acid protein lacking a membrane-anchoring domain and creating the fourth known potential soluble isoform of the α-subunit of the GM-CSF receptor. Am. J. Hematol. 58:145–147, 1998. © 1998 Wiley-Liss, Inc.

Key words: GM-CSF receptor; alternative splicing; juvenile myelomonocytic leukemia

INTRODUCTION

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is a potent hematopoietic growth factor that stimulates proliferation and maturation of myeloid progenitor cells. In addition, many of the primary myeloid leukemic blasts that form colonies in vitro are dependent on the presence of GM-CSF for proliferation. All these biological effects of GM-CSF are mediated by a specific cell surface receptor following GM-CSF binding. The human GM-CSF receptor is composed of at least two subunits (α and β). The α-subunit binds GM-CSF with low affinity, and the β-subunit does not bind GM-CSF by itself, but forms heterodimers with the α subunit, which results in a high-affinity receptor that is capable of transmitting growth signals [1]. Since the discovery of the first soluble isoform of the full-length α subunit (GM-CSFRα_1) by Ashworth and Kraft in 1991 [2], several other isoforms have been described [3–5]. Most of these isoforms are a result of alternative mRNA splicing at the 3′ end, which alters the coding region and hence the protein produced [2–5]. Two of them are the products of alternatively spliced forms of the 5′ untranslated region of the α-subunit mRNA [6]. We have previously reported two short variants of GM-CSFRα_1. One encodes a soluble isoform lacking exon 10 with 136 bp, and the other one has a 179-bp deletion corresponding to exons 11 and 12. The deletion resulted in a novel 62-aa carboxyl-terminus with an alternative carboxyl-terminal membrane-anchoring domain [5]. We report here another novel mRNA/cDNA variant of full-length of the α-subunit isolated from a patient with juvenile myelomonocytic myeloid leukemia (JMML). This cDNA has a 397-bp deletion (Fig. 1).

MATERIALS AND METHODS

Total RNA was isolated from 5 normal and 10 JMML patients from their mononuclear blood cells by the Gua-

Contract grant sponsor: National Cancer Institute; Contract grant number: CA56072.

*Correspondence to: Kenneth S. Zuckerman, M.D., Division of Medical Oncology and Hematology, University of South Florida College of Medicine, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612. E-mail: Zuckerman@moffitt.usf.edu

Received for publication 16 May 1997; Accepted 10 December 1997

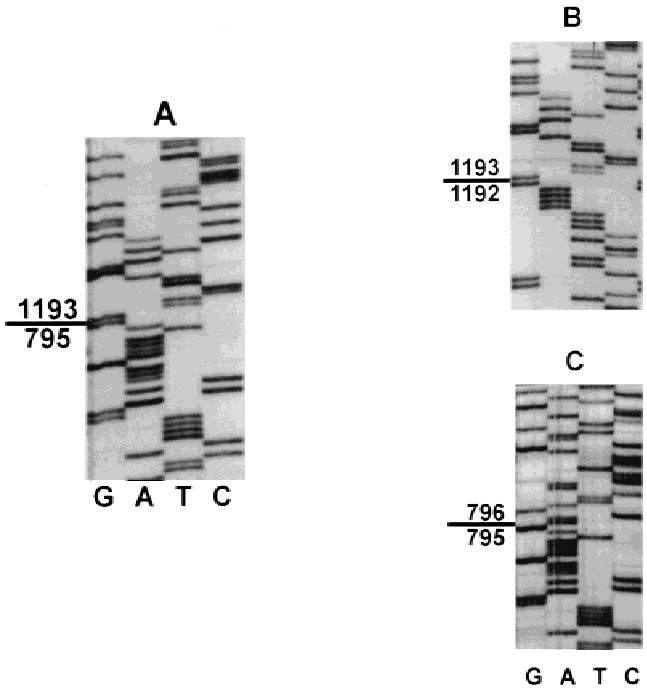

Fig. 1. Sequencing of the GM-CSFRα_1 (wild-type) and GM-CSFRα_{S3} (alternate splicing-derived variant). The sequence shows that GM-CSFRα_{S3} lacks the 397 base pairs from bp 796–1192 of the wild-type cDNA, which are the nucleotides encoded by exons 8, 9, 10, and 11. The sequence of the relevant portions of the wild-type GM-CSFRα_1 cDNA are shown in panels B and C, for purposes of comparison with the sequence of the relevant portion of the cDNA for GM-CSFRα_{S3} (panel A).

nidiuium thiocyanate method [7], and the RNA was then reverse transcribed using random hexamers (Gibco/PRL, Gaithersburg, MD). Full-length cDNA clones for the GM-CSF receptor α-subunit were isolated and amplified by PCR [5]. The oligos used for PCR are sense 5′-GCAAGTTCAAGCTTCCTTCGGCCTGTCGCT-3′ (nucleotides 106–125) and antisense 5′-GCAGTCAAG-TCTAGATGTCCATGCCATTCC-3′ (nucleotides 1385–1368). The sense oligo carries HindIII cleavage site, and the antisense carries XbaI cleavage site. The PCR products were cut with the restriction enzymes and ligated into expression vector pCDM8 (Invitrogen, San Diego, CA), and then transformed into *Escherichea coli* (MC1061/P3). The recombinant DNA was sequenced by the Sanger dideoxy chain termination method, using the Sequenase Version 2.0 kit (United States Biochemical Corp., Cleveland, OH).

RESULTS AND DISCUSSION

In addition to the expected full-length transcript, one small fragment was also detected from one patient. The sequence showed that the small transcript detected has a deletion of 397 bp corresponding to exons 8, 9, 10, and 11 of GM-CSFRα_1 cDNA (Fig. 1). This deletion causes a frame shift resulting in a cDNA that potentially encodes a 233 amino acid protein, and the cDNA does not encode any obvious membrane-anchoring domain. Therefore, this cDNA appears to encode a novel putatively soluble isoform of the receptor. The small fragment for this cDNA was not detectable by PCR in the samples from 5 normal persons.

PCR may cause a number of artifacts, such as single bp deletion or insertion. However, the following facts strongly suggest that the cDNA we isolated corresponds to a physiological mRNA: the deleted nucleotides for this novel cDNA and other variants we have described previously [5] always corresponded precisely to whole exons, and were only detectable in the patients, but not normal persons studied.

We have noticed that all of the exons deleted in this and several other cDNA variants of GM-CSFRα_1 [2,5] are located near the region encoding the membrane-spanning domain, indicating that this region might be an important site in physiological and pathophysiological regulation of the alternative splicing. It was also noticed that this and several other cDNA variants were isolated from leukemia patients [8] or leukemic cell lines [9], which suggests some pathologic conditions could lead to a high expression of alternative splicing isoforms.

RNA splicing has been a critical aspect of regulation of genes in eukaryotic cells. For example, each alternative splicing form of prostaglandin E receptor couples to one of several G proteins to activate a specific second message system [8]. Five splicing variants of pituitary adenylyl cyclase-activating polypeptide (PACAP) receptor also induce different signal transduction pathways [9]. Alternative splicing variants of Integrin β1 transmit distinct signals that markedly inhibit DNA synthesis [10]. Therefore, the information from studies of the GM-CSF receptor isoforms could be important in understanding the role of alternative splicing of mRNA in normal vs. neoplastic disorders. The sequence data reported in this paper have been deposited in the Genbank Data Libraries under accession number U93096.

ACKNOWLEDGMENTS

This work has been supported by USPHS grant CA56072 from the National Cancer Institute. We appreciate the technical assistance of Ariana Brown Fisher.

REFERENCES

1. Miyajima A, Mui A L-F, Ogorochi T, Sakamaki K: Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3 and interleukin-5. Blood 82:1960–1970, 1993.
2. Ashworth A, Kraft A: Cloning of a potentially soluble receptor for human GM-CSF. Nucleic Acids Res 18:7178, 1990.

3. Raines MA, Liu L, Quan SG, Joe V, Dipersio JF, Golde DW: Identification and molecular cloning of a soluble human granulocyte-macrophage colony-stimulating factor receptor. Proc Natl Acad Sci USA 88:8203–8207, 1991.
4. Crosier KE, Wong GG, Mathey-Prevot B, Nathan DG, Sieff CA: A functional isoform of the human granulocyte/macrophage colony-stimulating factor receptor has an unusual cytoplasmic domain. Proc Natl Acad Sci USA 88:7744–7748, 1991.
5. Hu X, Emanuel PD, Zuckerman KS: Cloning and sequencing of the cDNAs encoding two alternative splicing-derived variants of the alpha subunit of the granulacyte-macrophage colony-stimulating factor. Biochim Biophys Acta 1223:306–308, 1994.
6. Chopra R, Kendall G, Gale RE, Thomas NSB, Linch DC: Exp Hemat 24:755–762, 1996.
7. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159, 1987.
8. Namba T, Sugimoto Y, Negishi M, Irie A, Ushikuhi F, Kakizuka A, Ito S, Ichikawa A, Narumiya S: Alternative splicing of C-terminal tail of prostaglanding E receptor subtype EP3 determines G-protein specificity. Nature 365:166–170, 1993.
9. Spengler D, Waeber C, Pantaloni JC, Holsboer F, Bockaert J, Seeburg PH, Journot L: Differential signal transduction by five splice variants of the PACAP receptor. Nature 365:170–175, 1993.
10. Meredith J Jr, Takada Y, Fornaro M, Languino LR, Schwartz MA: Inhibition of cell cycle progression by the alternatively spliced integrin β_{1c}. Science 269:1570–1572, 1995.